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Short communication

A novel de novo mutation in *LAMB3* causes localized hypoplastic enamel in the molar region

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Amelogenesis imperfecta (AI) is a collection of diseases characterized by hereditary enamel defects and is heterogeneous in genetic etiology and clinical phenotype. In this study, we recruited a nuclear AI family with a proband having unique irregular hypoplastic pits and grooves in all surfaces of the deciduous molar teeth but not in the deciduous anterior teeth. Based on the candidate gene approach, we screened the laminin subunit beta 3 (*LAMB3*) gene and identified a novel de novo mutation in the proband. The mutation was a frameshift mutation caused by a heterozygous 7-bp deletion in the last exon (c.3452_3458delAGAAGCG, p.Glu1151Valfs*57). This study not only expands the mutational spectrum of the *LAMB3* gene causing isolated AI but also broadens the understanding of genotype–phenotype correlations.

Amelogenesis imperfecta (AI) is a collection of hereditary enamel defects that are heterogeneous in genetic etiology and clinical manifestation (1). Tooth enamel is the hardest calcified tissue in vertebrates, and its formation is under delicate control of a series of ectodermal–mesenchymal interactions (2). Any harmful influence, whether genetic or environmental, during amelogenesis could induce defects in the enamel. Frequently, an insult during development results in only enamel defects, indicating that amelogenesis is extremely vulnerable to such an event.

To date, more than 13 genes have been identified to cause AI. Even though the clinical phenotype is diverse and heterogeneous, some forms of AI share a common phenotype. Alternatively, there are some characteristic features related to a specific genetic etiology, enabling researchers to find the genetic cause based on the candidate gene approach (3). In the present study, we describe a family with AI with a unique pattern of irregular hypoplastic enamel pits and grooves. Mutational screening of the candidate gene successfully found a genetic etiology to associate genotype with phenotype.

Parents and a child with localized enamel hypoplasia visited the Department of Pediatric Dentistry, Seoul National University Dental Hospital. The parents and the child were informed of the study and agreed to participate. The study protocol was reviewed and approved by the Institutional Review Board of the Seoul National University Dental Hospital. Clinical examinations and blood collections were performed after obtaining written consent, according to the Declaration of Helsinki.

The proband was a 6-yr-old boy from non-consanguineous parents. Both parents were healthy without any systemic and/or oral conditions. The pregnancy and delivery were uneventful. The boy had no past history of systemic illness or skin pathology. His deciduous dentition showed unique irregular hypoplastic enamel pits and grooves in all surfaces of his deciduous molars, but not in his anterior teeth (Fig. 1A). In particular, second molars exhibited altered crown shape as a result of severe enamel hypoplasia (Fig. 1B-E). However, the thin enamel showed a distinct contrast with the underlying dentin, indicating that mineralization was not severely affected. The patient's developing teeth also exhibited irregular enamel hypoplastic features (Fig. 1F).

The exons, including the exon–intron boundaries of the laminin subunit beta 3 (*LAMB3*) gene, were sequenced as previously described (4). Sanger sequencing of exon 23, the last exon of the *LAMB3* gene, revealed a heterozygous 7-bp deletion (c.3452_3458delAGAAGCG, p.Glu1151Valfs*57) (Figure S1). This deletion caused a shift in the reading frame, resulting in a change from glutamine to valine at codon position 1151 and the production of an additional novel 56 amino acids (thus giving 1207 amino

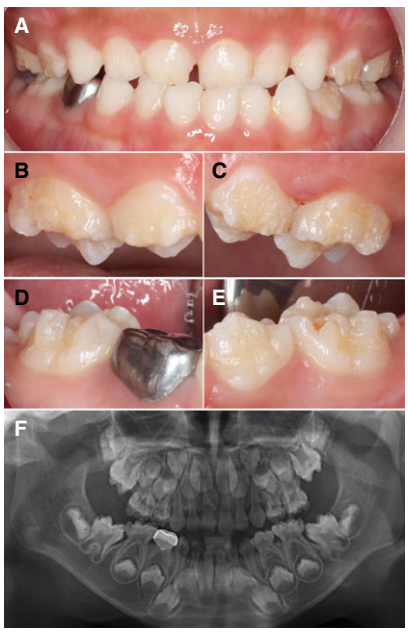

Fig. 1. Clinical photographs and panoramic radiograph of the proband. (A) Frontal clinical photograph of the proband. (B–E) Photographs showing occlusal and buccal surfaces of the deciduous molar teeth. All surfaces exhibit irregular hypoplastic enamel pits and grooves. The shape of the crown, especially that of the second deciduous molar, is altered as a result of severe enamel hypoplasia. (F) Panoramic radiograph of the proband. The irregular hypoplastic enamel shows a distinct contrast with the underlying dentin, indicating that enamel mineralization is not affected. The enamel of developing teeth also exhibits irregular hypoplasia.

acids in total), instead of the 1172-amino-acid wild-type LAMB3 protein. The parents were tested, but lacked the mutation, indicating that the mutation occurred spontaneously.

In this study, the proband exhibited enamel defects with unique irregular hypoplastic pits and grooves in all surfaces of primary molars but not in the anterior teeth. Hypoplastic AI can occur as a result of mutations in several genes: amelogenin, X-isoform (*AMELX*; MIM *300391) (5); enamelin (*ENAM*; MIM *606585) (6); family with sequence similarity 20 member A (*FAM20A*; MIM *611062) (7); integrin beta 6 (*ITGB6*; MIM *147558) (8); *LAMB3* (MIM *150310) (9–11); collagen type XVII alpha 1 (*COL17A1*; MIM *113811) (12); and laminin subunit alpha 3 (*LAMA3*; MIM *600805) (13).

Mutations in the *AMELX* gene are associated with X-linked hypoplastic and/or hypomaturation AI, and the clinical phenotype in male subjects is the generalized, rather than the localized, form of AI (1). Mutations in *ENAM* have been associated with generalized and localized hypoplastic enamel in an autosomal-dominant or autosomal-recessive manner. In the autosomal-recessive form, clinical severity has been shown to be related to the dosage of the mutant allele, and the carrier also exhibits some minor hypoplastic pits in several teeth (14). Frequently, horizontal hypoplastic grooves are a characteristic feature related to *ENAM* mutations (15). Autosomal-recessive *FAM20A* mutations result in generalized hypoplastic AI with gingival hyperplasia, multiple eruption failures, and renal calcification (7, 16). Autosomal-recessive *ITGB6* mutations result in generalized hypoplastic or pitted hypomineralized AI (8, 17).

Based on the clinical appearance of the unique irregular hypoplastic pits and grooves of the proband, we selected the *LAMB3* gene for candidate gene screening. Mutational screening of the *LAMB3* gene successfully identified a novel mutation. If the candidate screening had revealed no pathologic variation in the *LAMB3* gene, the next targets would have been the *COL17A1* and *LAMA3* genes as a result of the similar clinical enamel phenotype, which can occur without skin problems even though this is extremely rare (12, 13, 18).

The genes *LAMB3*, *LAMA3*, and *COL17A1* are all involved in the skin disease, junctional epidermolysis bullosa (JEB) (19). Junctional epidermolysis bullosa is a rare inherited disorder featuring skin fragility and AI from defects in the genes encoding the components of hemidesmosome/basement-membrane complexes (20). Junctional epidermolysis bullosa is usually manifested as an autosomal-recessive inheritance pattern; however, in some cases, a heterozygous mutation can cause autosomal-dominant AI with little or no apparent skin defect (12, 13, 18). The finding of a novel mutation in this study will bring the number of *LAMB3* mutations causing isolated AI to seven (Table S1). All seven *LAMB3* mutations causing isolated AI are truncating ones that can be expressed by escaping the nonsense-mediated mRNA decay system. The observed enamel defects seem not to be caused by haploinsufficiency because most JEB carriers do not have any dental phenotype. Disease-causing *LAMB3* truncation mutations will act in a dominant–negative manner to cause enamel defects affecting developmentally vulnerable amelogenesis, without skin pathology.

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Supporting Information

Additional Supporting Information may be found in the online version of this article:

Table S1. Disease-causing mutations in the *LAMB3* gene.

Figure S1. Sequencing chromatograms of the proband and unaffected parents.